

Assessing the Potential for Nitrogen Bubble Formation in Diving Odontocetes

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LONG-TERM GOALS

The goal of assessing whether nitrogen (N₂) bubble formation occurs in repetitively diving odontocetes is to address hypotheses related to the stranding of beaked whale species coincident with naval sonar activities. Specifically, data will be acquired that will either support or refute the conjecture that the accumulation of N₂ and subsequent formation of N₂ bubbles is a causative mechanism of beaked whale strandings.

OBJECTIVES

The objective of this study are to: 1) train a dolphin to repetitively dive a depth profile specifically created to maximize nitrogen absorption from the lung; 2) following a dive series, ultrasonically inspect the blood vessels of the dolphin for the presence of nitrogen bubbles; 3) determine the partial pressure of N₂, or PN₂, in arterialized venous blood following a diver series; and 4) relate the partial pressure of N₂ to the presence or absence of vascular bubbles.

APPROACH

A trained dolphin from the Navy Marine Mammal Program (MMP) was used for the study. Some differences between beaked whales and delphinoids in their respective physiology and anatomy are acknowledged; however, no other surrogate species were readily available or amenable for the proposed testing.

The subject was transported via a modified Boston whaler to a deep-water site where it was trained to perform a series of dives to depths between 10 and 100 m. A stationing platform, fitted with an underwater video camera and a biteplate (for the dolphin to bite onto), was lowered to the desired dive depth. At the beginning of a dive series, the dolphin was cued by an animal trainer to descend to the dive platform. At the bottom of each dive, the dolphin stationed on the biteplate for a period of ~ 1.5 minute. Stationing at the bottom of the dive was verified by the underwater video camera. The subject was cued to leave from the biteplate by an underwater sound projector placed approximately 1 m below the surface. The subject performed 10-14 dives per trial and one trial was conducted per day. Each dive was conducted in sequence with surface intervals between dives being limited to ~ 1 minute. Dive behavior was recorded throughout each session by the use of a time-depth recorder, which was worn on the pectoral fin of the dolphin.

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Previous modeling (Houser et al. 2001) suggested that N₂ tension of the muscle at the end of this dive sequence would approach 350%. The modeling did not account for exchange between compartments of the body (i.e., tissues with different N₂ solubility) and continued distribution of the N₂ load at depth, and likely overestimated the N₂ gas saturation of the muscle bed. Nevertheless, given that the first compartment within which exchange occurs is the blood pool, and assuming that cetacean hematological factors do not inhibit bubble formation, it was expected that blood N₂ saturation exceeded 300% and provided sufficient over-saturation for bubble formation to occur. (In terrestrial mammals, differentials of ~2 ATM between tissue and ambient partial pressures have been observed to result in bubble formation; Gersh et al., 1944.)

At the end of the dive sequence the subject was ultrasonically tested for the presence of vascular bubbles using a Sonosite TITAN portable ultrasound unit. Ultrasound inspections were conducted with the animal out of the water and in the water by the side of the boat. For out-of-water inspections, the dolphin beached itself into a padded transport mat within the boat. The portal (Figure 1) and innominate veins were investigated for the presence of bubbles after the dive series and were inspected for durations of 1-10 minutes. The portal and innominate veins were selected because of the relationship between “bubble-like” cavitary lesions reported in the portal system of some stranding cetaceans, and the accessibility of these vessels to ultrasound inspection.

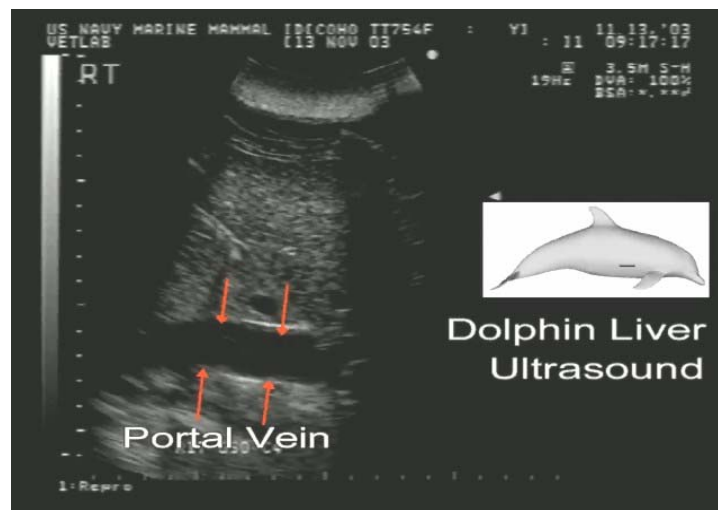


Figure 1. Ultrasound image of the portal vein of a bottlenose dolphin (designated by the red arrows). The insert image of the dolphin shows from where on the dolphin the ultrasound image was acquired (indicated by the horizontal bar behind the pectoral fin).

The SonoSite TITAN portable ultrasound was used in conjunction with a C15 transducer, which operates from 2 – 4 MHz, has a penetration depth of 24.6 cm, and a field of view of 101 degrees. Bubble detection was attempted with both pulsed wave (PW) Doppler and 2D visual modes. Intravascular bubbles have traditionally been assessed following decompression through the use of Doppler flow transducers. Because the Doppler frequency shift from scatterers is within the audible range of hearing, and because sound scattering is more effective from bubbles than from blood cells (~99.9% of incident wave energy), the Doppler method allows bubble formation to be graded by a listener (see Nishi et al., 2003 for examples). In contrast, the 2D visual mode permitted visual

identification and video capture of intravascular bubbles as transient specular reflections in the field of view.

At the end of one dive sequence to 50, 70 or 100 m, serial voluntary blood samples were taken from the fluke of the dolphin over a 20-min period (Figure 2). Collected blood samples were used to assess blood N₂ levels. Nitrogen partial pressures (PN₂) were determined with a Van Slyke technique. Post-dive blood oxygen and carbon dioxide partial pressures (PO₂ and PCO₂) were determined with an I-stat portable blood analyzer after the 70- and 100-m dive series.

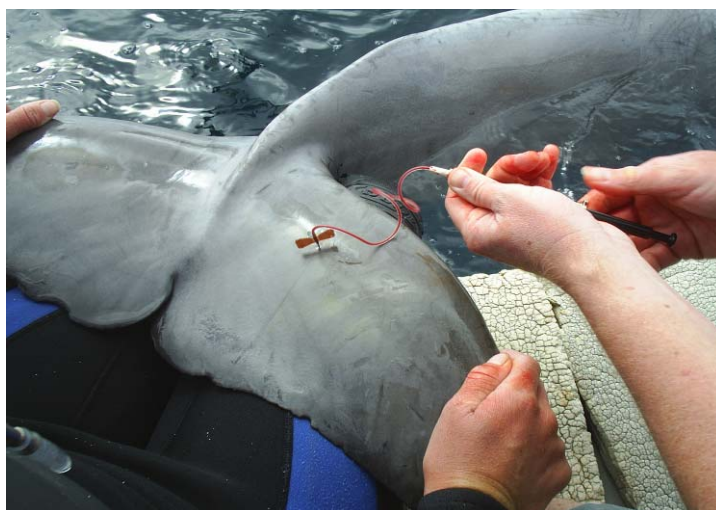


Figure 2. Blood sample being collected from a fluke vessel of the dolphin following a sequence of dives to depth.

Dr. Dorian Houser served as the PI on project and was responsible for facilities coordination, animal welfare issues, ultrasound inspections and blood collections from the animal. Ms. Lois Dankiewicz served as the daily manager of project personnel and coordinated boat use, training plans, data archiving, and personnel development. A number of trainers (Elizabeth Testa, Jennifer Sellers, Elizabeth Piotrowski, Jennifer Hanson and Taylor Jensen) were involved with the training of the dolphin and assistance in the deployment of dive station equipment and data collection. The following undergraduate volunteers were utilized as interns on this project: Nanette Duarte, Elizabeth Heppe, Whittaker Campbell, Brian Mauer and Jillian Lai.

WORK COMPLETED

A dolphin was trained to perform repetitive dives to depths of 10, 30, 50, 70 and 100 m. A total of 20 ultrasound inspections were successfully completed of the portal and innominate veins. Blood collections for assessment of PN₂ were made once for each of the 50, 70 and 100 m dive series. All project objectives were completed. In addition, collaborative efforts to attempt an assessment of dolphin lung collapse and dolphin diving heart rate measurements were completed (see Related Projects).

RESULTS

No vascular bubbles were observed in any of the post-dive ultrasound inspections. Post-dive blood PN₂ values were not significantly elevated above those of dolphins at rest (Figure 3). Average PCO₂ and PO₂ of blood samples were 48 (+ 4.1) and 59 (+ 2.6) mm Hg, respectively.

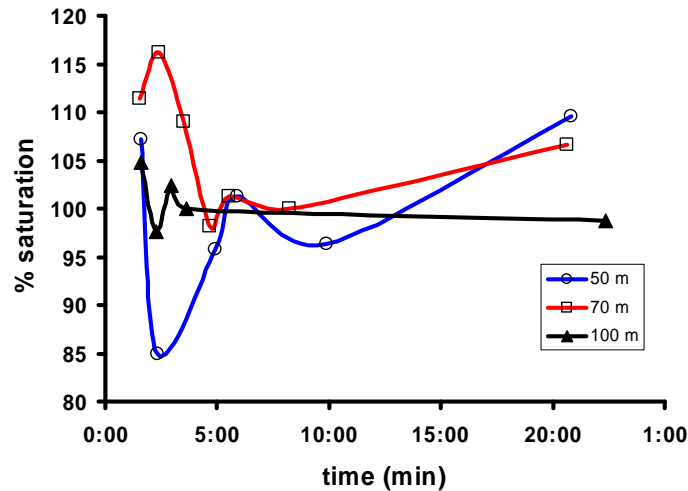


Figure 3. Variation in the PN₂ of arterialized-venous blood samples collected following 10 dives to depths of 50, 70 and 100 m. The N₂ saturation of the samples is not substantially different from baseline values.

Ultrasound results suggest that for bouts of repetitive, prolonged dives up to 100-m depth, the accumulation of N₂ is insufficient to generate asymptomatic intravascular bubbles in bottlenose dolphins. The post-dive PN₂ data similarly reflect minimal N₂ accumulation in fluke tissue during dives. The blood PO₂ and PCO₂ data, consistent with arterialized venous blood samples, also suggest efficient pulmonary clearance of blood N₂ at the surface.

IMPACT/APPLICATIONS

If the depth of lung collapse in beaked whales is similar to that in dolphins, then the lack of bubble formation and elevated PN₂ observed in this study do not support the hypothesis that alveolar N₂ uptake is sufficient to be an etiology for beaked whale strandings coincident with SONAR exposure. However, since blood samples were only obtainable from arterialized venous blood in the periphery (the fluke), definitive N₂ washout curves from the body core (central organs and muscle) still await post-dive central venous blood sampling (assuming development of appropriate blood collection techniques). Furthermore, the impact of slow exchanging, high N₂ solubility compartments (e.g. bone marrow) on the retention of N₂ in lower solubility compartments is undetermined and may be an important aspect of N₂ kinetics for beaked whales diving below the depth of lung collapse.

RELATED PROJECTS

Detection of dolphin lung collapse via acoustic backscatter – Assumptions about the depth of lung collapse in diving odontocetes are critical to assessments of N₂ accumulation while diving since N₂ accumulation ceases below the depth of lung collapse. In collaboration with Dr. Whitlow Au of the University of Hawaii, an attempt was made to determine the depth of lung collapse in the dolphin used in this project. The approach consisted of looking at the backscatter of the dolphin ensonified with 70, 120 and 200 kHz echosounder pulses during the performance of the dolphin's dive sequence. Due to an inability to control the exact orientation of the dolphin or maintain its position within the echosounder beams while diving, an accurate assessment of lung collapse was not attainable. Results of this collaborative effort have been presented at the Acoustical Society of America (Au, W. W. L., Houser, D. S. and Dankiewicz, L. A. 2007. Acoustic backscatter from a diving dolphin. 153rd Meeting of the Acoustical Society of America).

Dolphin cardiac function during deep, repetitive diving – A collaborative effort with Drs. Paul Ponganis and Torre Stockard of the Scripps Institute of Oceanography was undertaken to assess the cardiac function of the subject during deep and repetitive diving. Cardiac output will directly affect the distribution of the gas load carried by the blood (N₂, O₂, CO₂) and thus affects gas kinetics within the tissues of the dolphin. The SIO collaborators developed a heart rate monitor that was worn by the dolphin while diving. Results obtained from the effort will be combined with the vascular bubble study for publication.

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PUBLICATIONS

Houser, D. S., Dankiewicz, L. A., Stockard, T. K. and Ponganis, P. J. Ultrasound inspection for intravascular bubbles in a repetitively diving dolphin. *Bioacoustics* [in press].

HONORS/AWARDS/PRIZES

Dorian S. Houser, *Biomimetica* – 'R. Bruce Lindsay Award' given by the Acoustical Society of America (2007)

Dorian S. Houser, *Biomimetica* – 'Outstanding Young Alumni Award' given by Coker College (2006)